Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.)*

U. SINGH and R. JAMBUNATHAN

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), ICRISAT Patancheru PO 502 324, Andhra Pradesh, India

(*Received 6 July 1981; in revised form 19 February 1982*)

Key words: amino acids, chickpea, pigeonpea, protein fractions, seed

Abstract. Studies on protein fractionation in seed coat, embryo, cotyledons and whole seed were made to observe the differences, if any, between chickpea and pigeonpea. Results indicated that globulin was the major fraction of embryo and cotyledons of these legumes. Seed-coat nitrogen was observed to be mostly comprised of nonprotein nitrogen and glutelin fractions and thus differed from other components in both chickpea and pigeonpea. The albumin fraction of cotyledons of both crops had the highest concentration of sulphur amino acids, methionine and cystine. Glutelin contained a considerably higher concentration of methionine and cystine than did globulin in chickpea and pigeonpea. This suggests that lines with higher glutelin should be identified to improve their protein quality. The amino acid compositions of different seed components did not show large differences between these two pulse crops.

Introduction

Chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.) are the two most important food legumes in India and they provide additional protein and calories in the largely cereal-based diet of the people. In general, the protein quality of these grain legumes is primarily limited by their low levels of essential amino acids, methionine, cystine and tryptophan [3, 4]. The proteins present in legume seeds can be broadly classified into metabolic proteins, which are involved in normal cellular activities, and storage proteins, which are synthesised during seed development. The storage protein, globulin, constitutes a major proportion of the legume seed proteins and the limitations of these proteins in the nutrition of humans and other monogastric animals are well known [7]. The amino acid composition of food crops can be altered either by varying the relative proportions of embryo and endosperm or by changing the relative proportions of metabolic and storage proteins as in the case of opaque-2 maize [6].

Earlier, workers reported the distribution of nitrogen, mineral and trace elements in the various anatomical parts of commonly consumed Indian pulse crops [9]. Pant et al. [8] fractionated the seed flour of 28 species of non-

* Submitted as JA no. 180 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

edible legumes into fractions such as nonprotein nitrogen, albumin, globulin, prolamin and glutelin. Fractionation and amino acid composition of bean cotyledon protein has revealed that the alkali-soluble fraction has the highest concentration of methionine [12]. Abdi and Sahib [1] reported that most of the lysine of horse gram (*Dolichos biflorus*) seed is present in the albumin fraction. Amino acid analysis by a paper chromatography technique indicated that the water-soluble and alkaline-soluble fractions of chickpea were larger in cystine, lysine, methionine and tryptophan, while the salt-soluble fraction was found to be rich in arginine and glutamic acid [5]. Information on the distribution of seed protein fractions and amino acids in food legumes is limited. The results of chickpea and pigeonpea protein fractionation studies, the amino acid composition of the protein fractions, and the amino acid composition of different seed components of the pulse crops are reported in this article.

Materials and Methods

Materials. Pigeonpea (cv. Hy-3c) and chickpea (cv. G-130) were grown in rainy and post-rainy seasons of 1978–79, respectively, and were supplied by our breeding program. Seed coat was separated from the whole grain manually after soaking the seed material at 4°–5°C for 4 h. Embryo was separated from the cotyledons by hand dissection using a needle. The different components were dried in an oven at 65°C and samples were ground to a fine powder in a Udy cyclone mill using a 0.4-mm screen. The samples were defatted in a Soxhlet apparatus using hexane.

Separation of different protein fractions. The separation of different protein fractions was carried out using the procedure described earlier [11]. The protein extracts containing albumin and globulin in 0.5 *M* sodium chloride solution in 0.01 *M* phosphate buffer (pH 7.0) were dialysed against six changes of distilled water at room temperature (25°C) for 72 h and the volume was made to 50 ml. The dialysate was then centrifuged (12 000 *g* for 15 min) and the pellet and supernatant of the dialysate were referred to as the globulin and albumin, respectively. However, nonprotein nitrogen was lost during the process. These fractions were analysed for nitrogen and then freeze dried.

Total nitrogen and nonprotein nitrogen. The nitrogen content of the protein fractions and other seed components was determined using a micro-Kjeldahl procedure [2] and protein was calculated (N × 6.25). Nonprotein nitrogen (NPN) was estimated by extraction of the samples with 10% trichloroacetic acid (TCA) as described earlier [10].

Amino acid analysis. The amino acid profiles of the freeze-dried protein fractions of cotyledons and different seed components were determined using a

Beckman 120-C amino acid analyser. Acid hydrolysis was performed by refluxing for 24 h with constant-boiling 6 *N* HC1. The excess acid was removed in a flash evaporator and made up to volume using citrate buffer (pH 2.2) before analysis. In the case of pigeonpea, mean coefficient of variability of analysis for different amino acids ranged between 1.3% and 9.0% except for isoleucine and histidine, where it was 11.3% and 11.9%, respectively; whereas limits of reproducibility for different amino acids varied between 2% and 10% in case of chickpea.

Results and Discussion

Distribution of protein fractions

The results on distribution of protein fractions in different seed components of chickpea and pigeonpea are summarised in Table 1. Both chickpea and pigeonpea are made of three anatomical structures: the seed coat, the cotyledons and the embryonic tissue [9]. Embryos constitute only a small proportion of the total seed weight whereas the cotyledons constitute 82.9% and 85.3% of total dry weight in chickpea and pigeonpea, respectively (Table 1). These values agree with earlier reported values [9]. Embryo and seed-coat contents were slightly higher in chickpea than in pigeonpea. Protein fractionation of seed coats, embryos, cotyledons, and whole seeds of chickpea and pigeonpea did not reveal large differences between these two legumes (Table 1), but considerable differences in the distribution pattern of protein fractions among the embryo, cotyledons and seed coats of these two legumes were observed. When compared with other components, the embryo was found to be richer in albumin both in chickpea and in pigeonpea. Whole-seed chickpea had a slightly lower concentration of globulin than pigeonpea. Non-protein nitrogen and glutelin fractions were higher in the seed coat as compared to other components and they had a much smaller proportion of albumin and globulin fractions.

Amino acid composition of different protein fractions

Having observed that the cotyledons accounted for about 80%–85% of the total dry-seed weight, various protein fractions of this component were analysed for amino acid composition and the results are shown in Table 2. When the amino acid profile of different fractions was compared, albumin was noticed to have the largest amount of sulphur amino acids, methionine and cystine, lysine, aspartic acid, glycine and alanine in the case of both chickpea and pigeonpea. This fraction has also been identified as a rich source of lysine in horse gram [1]. By calculation it was observed that this fraction contributed about 36% and 35% of the toal sulphur amino acids of the cotyledons of chickpea and pigeonpea, respectively.

Globulin, the major protein fraction, had lower methionine and cystine

Table 1. Distribution of protein fractions in different components of chickpea and pigeonpea

Crop/component	Amount[a] (%)	Protein[a] (%) (N × 6.25)	Nonprotein nitrogen[b] (%)	Protein fractions[b]					
				Albumin	Globulin	Glutelin	Prolamin	Residue	Total
Chickpea									
Embryo	1.2	52.1	5.8	22.5	50.0	21.4	3.0	1.5	98.4
Cotyledon	82.9	24.8	10.7	15.9	62.7	17.5	2.3	1.0	99.4
Seed coat	16.4	4.1	21.3	3.5	22.8	33.2	3.4	30.5	93.4
Whole seed	–	21.3	11.2	12.6	56.6	18.1	2.8	4.9	95.0
Pigeonpea									
Embryo	0.7	49.6	6.2	17.0	52.7	21.3	2.7	2.1	95.8
Cotyledon	85.3	22.2	9.5	11.4	64.5	18.2	3.5	1.8	99.4
Seed coat	14.3	4.9	27.4	2.6	26.3	32.8	4.2	23.0	88.9
Whole seed	–	20.5	12.8	10.2	59.9	17.4	3.0	5.3	95.8

[a] Dry weight

[b] Values are averages of two determinations and expressed as percentage of total protein (N × 6.25)

Table 2. Amino acid composition (g/16 g N) of seed protein fractions of chickpea and pigeonpea cotyledons

	Chickpea				Pigeonpea			
Amino acid	Albumin	Globulin	Glutelin	Prolamin	Albumin	Globulin	Glutelin	Prolamin
Lysine	10.8	6.4	6.8	2.3	10.0	6.9	7.1	1.0
Histidine	2.3	2.6	2.9	2.6	3.5	3.4	4.3	1.0
Arginine	5.6	10.7	6.8	4.8	6.4	7.0	7.6	1.3
Aspartic acid	13.8	12.7	10.1	10.3	13.9	10.8	11.8	3.9
Threonine	5.4	3.5	5.7	2.2	6.0	3.6	5.2	0.6
Serine	5.2	5.2	5.6	1.9	6.1	4.7	6.0	1.0
Glutamic acid	18.4	15.2	16.6	17.7	24.3	22.0	25.1	15.9
Proline	4.9	5.2	4.8	7.2	4.8	3.3	7.1	2.3
Glycine	5.4	3.7	4.7	3.1	5.9	4.0	4.8	1.3
Alanine	5.3	4.3	4.9	2.3	7.2	4.1	5.7	1.3
Cystine	3.5	1.0	1.4	0.6	3.2	0.9	1.0	0.4
Valine	4.5	4.2	5.7	2.1	6.2	5.5	5.8	1.8
Methionine	1.8	0.8	1.2	0.9	1.7	0.8	1.3	0.3
Isoleucine	5.1	4.4	5.4	2.3	4.1	3.4	5.1	0.7
Leucine	9.8	7.5	9.1	1.6	7.7	6.7	9.2	0.8
Tyrosine	4.2	2.9	3.7	2.3	4.2	3.9	3.9	0.9
Phenylalanine	5.1	6.1	4.4	3.4	4.7	10.9	8.0	6.5
Total	111.1	96.4	99.8	64.6	119.9	101.9	119.0	41.0
N recovery (%)	95.7	90.5	87.5	54.2	98.8	84.6	96.5	29.3

contents than the glutelin fraction. Since methionine is one of the limiting essential amino acids of these legumes, a larger proportion of protein fractions containing this amino acid would be advantageous from the nutritional viewpoint. The results obtained suggest that the selection of cultivars in which the albumin or glutelin fraction is present in higher proportions would result in improved methionine content in the whole seed.

Chickpea and pigeonpea differed from each other with respect to the amino acid profile of prolamin fraction. In the case of pigeonpea, this fraction had the highest amount of glutamic acid, followed by phenylalanine; whereas aspartic acid and glutamic acid were the predominant amino acids of this fraction in chickpea. Nitrogen recovery values were the lowest for these two prolamin fractions. When expressed on an equal nitrogen recovery basis, they had the poorest lysine of all other fractions.

Amino acid composition of different seed components

Amino acid profiles of whole-seed, embryo, cotyledon and seed-coat samples of chickpea and pigeonpea are shown in Table 3. Amino acid composition of cotyledons revealed some noticeable differences between chickpea and pigeonpea. Levels of lysine, glutamic acid, and phenylalanine were higher in pigeonpea than in chickpea. But the reverse was the trend for aspartic acid and sulphur-containing amino acids. Differences in the amino acid composition of the cotyledons will affect the overall nutritional potential of these legumes since cotyledons constitute a major proportion of the whole seed. Amino acid composition of embryos was observed to be nutritionally better than that of the cotyledons in both chickpea and pigeonpea as these contained higher amounts of lysine and sulphur amino acids. Levels of other amino acids of embryos were very similar to those of their respective cotyledons. Seed coats of chickpea and pigeonpea showed amino acid compositions slightly different from those of embryos and cotyledons. The relative proportions of serine, threonine, proline and glycine appeared to be considerably larger in seed coat than that in cotyledons in both chickpea and pigeonpea, when expressed on an equal nitrogen recovery basis.

Summary

The distribution of various anatomical parts of seeds did not reveal large differences between chickpea and pigeonpea. While no noticeable differences between chickpea and pigeonpea are apparent with respect to the levels of various protein fractions, the higher levels of sulphur-containing amino acids in glutelin than in globulins of these pulse crops suggest that cultivars with a higher ratio of glutelin to globulin should be identified to improve their seed protein quality.

Acknowledgement. The technical assistance of R. Seetha and Santosh Gurtu in analysing samples for amino acid composition is gratefully acknowledged.

Table 3. Amino acid composition (g/16 g N) of different seed components of chickpea and pigeonpea

	Chickpea				Pigeonpea			
Amino acid	Whole seed	Embryo	Cotyledons	Seed coat	Whole seed	Embryo	Cotyledons	Seed coat
Lysine	6.2	7.9	6.7	5.0	6.8	7.0	7.1	3.9
Histidine	2.7	2.6	2.7	2.4	3.6	3.3	3.9	1.0
Arginine	10.9	10.3	10.8	4.2	6.3	8.4	7.1	3.2
Aspartic acid	12.2	10.4	11.8	9.0	10.4	10.1	11.0	5.7
Threonine	4.0	4.5	3.8	3.7	3.8	4.7	4.3	2.5
Serine	5.5	5.0	5.3	4.7	5.0	5.3	5.5	3.5
Glutamic acid	16.3	17.6	16.1	10.7	19.0	16.2	20.6	6.9
Proline	4.0	2.6	3.9	3.9	4.3	4.7	4.3	3.1
Glycine	4.1	4.6	3.9	4.3	3.8	4.5	4.0	4.5
Alanine	4.0	5.1	4.2	3.9	4.6	6.0	4.5	3.0
Cystine	1.3	1.5	1.5	1.1	1.2	1.7	1.3	—
Valine	5.0	5.1	4.8	5.2	4.4	6.6	5.6	3.2
Methionine	1.1	1.5	1.1	1.1	1.0	1.4	1.2	0.7
Isoleucine	4.5	4.1	4.2	3.5	3.9	4.4	4.3	2.9
Leucine	7.6	7.4	7.2	6.3	7.2	7.1	7.8	4.0
Tyrosine	2.8	3.2	2.7	2.4	3.0	3.8	3.2	1.7
Phenylalanine	5.5	4.3	5.5	4.6	9.7	6.8	10.7	2.3
Total	97.7	97.7	96.2	76.0	98.0	102.0	106.4	52.1
N recovery (%)	91.5	88.5	89.1	54.4	92.5	94.5	97.4	46.9

References

1. Abdi H, Sahib MK (1976) Distribution of lysine in different legumes and some species of Amaranthus seeds. J Food Sci Technol 13:237–239
2. Association of Official Analytical Chemists (1975) Official methods of analysis, 12th edn. Washington DC: AOAC
3. Bressani R (1972) Legumes in human diets and how they might be improved. In: Nutritional improvement of food legumes by breeding, Proc Symp PAG/FAO, Rome, Italy
4. Food and Agriculture Organization (1970) Amino acid contents of foods and biological data on proteins. Rome: Food Policy and Food Science Service, Nutrition Division, FAO
5. Mathur KS, Sharma RD, Ram N (1968) Chromatographic amino acid pattern of various protein fractions of Bengal gram. Ind J Med Res 56:863–865
6. Mertz ET, Bates LS, Nelson OE (1964) Mutant gene that changes protein and increase lysine content of maize endosperm. Science 145:279–280
7. Millerd A (1975) Biochemistry of legume seed proteins. Ann Rev Plant Physiol 26:53–72
8. Pant R, Nair CR, Singh KS, Koshti GS (1974) Amino acid composition of some wild legumes. Curr Sci 43:235–239
9. Singh S, Singh HD, Sikka KG (1968) Distribution of nutrients in the anatomical parts of common Indian pulses. Cereal Chem 43:13–18
10. Singh U, Jambunathan R (1981) Relationship between total nitrogen and nonprotein nitrogen in chickpea (*Cicer arietinum* L.) seed. J Agric Food Chem 29:423–425
11. Singh U, Jambunathan R, Gurtu S (1981) Seed protein fractions and amino acid composition of some wild species of pigeonpea. J Food Sci Technol 18:83–85
12. Yu Ma, Bliss FA (1978) Seed proteins of common bean. Crop Sci 17:431–437